PCT

REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.

For receiving Office use only

International Application No.

International Filing Date

Name of receiving Office and "PCT International Application"

Applicant's or agent's file reference *(if desired) (12 characters maximum)* H1875 PCT S3

Box No. I TITLE OF INVENTION

STEROID MODIFIED SOLATRIOSES

Box No. II APPLICANT ☐ This person is also inventor

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

GLYCOMED SCIENCES LIMITED
P.O. Box 115
Turramurra NSW 2074
AU

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Facsimile No.

Teleprinter No.

Applicant's registration No. with the Office

State *(that is, country)* of nationality: AU

State *(that is, country)* of residence: AU

This person is applicant for the purposes of: ☐ all designated States ☒ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

LAWSON, Christopher John
Dextra Laboratories, Ltd.
Earley Gate, Whiteknights Road
Reading RG6 6BZ
GB

This person is:
☐ applicant only
☒ applicant and inventor
☐ inventor only *(If this check-box is marked, do not fill in below.)*

Applicant's registration No. with the Office

State *(that is, country)* of nationality: GB

State *(that is, country)* of residence: GB

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

☒ Further applicants and/or (further) inventors are indicated on a continuation sheet.

Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as: ☒ agent ☐ common representative

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

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Teleprinter No.

Agent's registration No. with the Office

☐ **Address for correspondence:** Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

Continuation of Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

If none of the following sub-boxes is used, this sheet should not be included in the request.

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

WEYMOUTH-WILSON, Alexander Charles
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This person is:
- [ ] applicant only
- [x] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

Applicant's registration No. with the Office

State *(that is, country)* of nationality: GB

State *(that is, country)* of residence: GB

This person is applicant for the purposes of: [ ] all designated States [ ] all designated States except the United States of America [x] the United States of America only [ ] the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

This person is:
- [ ] applicant only
- [ ] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

Applicant's registration No. with the Office

State *(that is, country)* of nationality:

State *(that is, country)* of residence:

This person is applicant for the purposes of: [ ] all designated States [ ] all designated States except the United States of America [ ] the United States of America only [ ] the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

This person is:
- [ ] applicant only
- [ ] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

Applicant's registration No. with the Office

State *(that is, country)* of nationality:

State *(that is, country)* of residence:

This person is applicant for the purposes of: [ ] all designated States [ ] all designated States except the United States of America [ ] the United States of America only [ ] the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

This person is:
- [ ] applicant only
- [ ] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

Applicant's registration No. with the Office

State *(that is, country)* of nationality:

State *(that is, country)* of residence:

This person is applicant for the purposes of: [ ] all designated States [ ] all designated States except the United States of America [ ] the United States of America only [ ] the States indicated in the Supplemental Box

[ ] Further applicants and/or (further) inventors are indicated on another continuation sheet.

Box No. V DESIGNATIONS

The filing of this request **constitutes under Rule 4.9(a), the designation** of all Contracting States bound by the PCT on the international filing date, for the grant of every kind of protection available and, where applicable, for the grant of both regional and national patents.

However,

☐ DE Germany **is not designated** for any kind of national protection

☐ KR Republic of Korea **is not designated** for any kind of national protection

☐ RU Russian Federation **is not designated** for any kind of national protection

(The check-boxes above may be used to exclude (irrevocably) the designations concerned in order to avoid the ceasing of the effect, under the national law, of an earlier national application from which priority is claimed. See the Notes to Box No. V as to the consequences of such national law provisions in these and certain other States.)

Box No. VI PRIORITY CLAIM

The priority of the following earlier application(s) is hereby claimed:

Filing date of earlier application *(day/month/year)*	Number of earlier application	Where earlier application is:		
		national application: country or Member of WTO	regional application:* regional Office	international application: receiving Office
item (1) July 8, 2003	03 01 5501.4		EP	
item (2)				
item (3)				

☐ Further priority claims are indicated in the Supplemental Box.

The receiving Office is requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) *(only if the earlier application was filed with the Office which for the purposes of this international application is the receiving Office)* identified above as:

☐ all items ☒ item (1) ☐ item (2) ☐ item (3) ☐ other, see Supplemental Box

** Where the earlier application is an ARIPO application, indicate at least one country party to the Paris Convention for the Protection of Industrial Property or one Member of the World Trade Organization for which that earlier application was filed (Rule 4.10(b)(ii)):* ....

Box No. VII INTERNATIONAL SEARCHING AUTHORITY

Choice of International Searching Authority (ISA) *(if two or more International Searching Authorities are competent to carry out the international search, indicate the Authority chosen; the two-letter code may be used)*:

ISA / EPO

Request to use results of earlier search; reference to that search *(if an earlier search has been carried out by or requested from the International Searching Authority)*:

Date *(day/month/year)* Number Country *(or regional Office)*

Box No. VIII DECLARATIONS

The following **declarations** are contained in Boxes Nos. VIII (i) to (v) *(mark the applicable check-boxes below and indicate in the right column the number of each type of declaration)*:		Number of declarations
☐ Box No. VIII (i)	Declaration as to the identity of the inventor :	
☐ Box No. VIII (ii)	Declaration as to the applicant's entitlement, as at the international filing date, to apply for and be granted a patent :	
☐ Box No. VIII (iii)	Declaration as to the applicant's entitlement, as at the international filing date, to claim the priority of the earlier application :	
☐ Box No. VIII (iv)	Declaration of inventorship (only for the purposes of the designation of the United States of America) :	
☐ Box No. VIII (v)	Declaration as to non-prejudicial disclosures or exceptions to lack of novelty :	

Box No. IX CHECK LIST; LANGUAGE OF FILING

This international application **contains:**

(a) **in paper form,** the following number of sheets:

request (including declaration sheets)	:	4
description (excluding sequence listing and/or tables related thereto)	:	15
claims	:	9
abstract	:	1
drawings	:	
Sub-total number of sheets	:	29
sequence listing	:	
tables related thereto	:	
(for both, actual number of sheets if filed in paper form, whether or not also filed in computer readable form; see (c) below)		
Total number of sheets	:	29

(b) ☐ **only in computer readable form** (Section 801(a)(i))
- (i) ☐ sequence listing
- (ii) ☐ tables related thereto

(c) ☐ **also in computer readable form** (Section 801(a)(ii))
- (i) ☐ sequence listing
- (ii) ☐ tables related thereto

Type and number of carriers (diskette, CD-ROM, CD-R or other) on which are contained the
- ☐ sequence listing: . . . . . .
- ☐ tables related thereto: . . . . . .

(additional copies to be indicated under items 9(ii) and/or 10(ii), in right column)

This international application is **accompanied by** the following item(s) *(mark the applicable check-boxes below and indicate in right column the number of each item):*

	Number of items
1. ☐ fee calculation sheet	:
2. ☐ original separate power of attorney	:
3. ☐ original general power of attorney	:
4. ☐ copy of general power of attorney; reference number, if any:	:
5. ☐ statement explaining lack of signature	:
6. ☐ priority document(s) identified in Box No. VI as item(s):	:
7. ☐ translation of international application into *(language)*:	:
8. ☐ separate indications concerning deposited microorganism or other biological material	:
9. ☐ sequence listing in computer readable form *(indicate type and number of carriers)*	
(i) ☐ copy submitted for the purposes of international search under Rule 13*ter* only (and not as part of the international application)	:
(ii) ☐ *(only where check-box (b)(i) or (c)(i) is marked in left column)* additional copies including, where applicable, the copy for the purposes of international search under Rule 13*ter*	:
(iii) ☐ together with relevant statement as to the identity of the copy or copies with the sequence listing mentioned in left column	:
10. ☐ tables in computer readable form related to sequence listing *(indicate type and number of carriers)*	
(i) ☐ copy submitted for the purposes of international search under Section 802(b-*quater*) only (and not as part of the international application)	:
(ii) ☐ *(only where check-box (b)(ii) or (c)(ii) is marked in left column)* additional copies including, where applicable, the copy for the purposes of international search under Section 802(b-*quater*)	:
(iii) ☐ together with relevant statement as to the identity of the copy or copies with the tables mentioned in left column	:
11. ☐ other *(specify)*:	:

Figure of the drawings which should accompany the abstract:

Language of filing of the international application: English

Box No. X SIGNATURE OF APPLICANT, AGENT OR COMMON REPRESENTATIVE

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request).

Dr. Rudolf Weinberger
European Patent Attorney

Vossius & Partner
Siebertstr. 4
81675 München
(Nr. 31)

For receiving Office use only

1. Date of actual receipt of the purported international application:

2. Drawings:
☐ received:
☐ not received:

3. Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application:

4. Date of timely receipt of the required corrections under PCT Article 11(2):

5. International Searching Authority (if two or more are competent): ISA /

6. ☐ Transmittal of search copy delayed until search fee is paid

For International Bureau use only

Date of receipt of the record copy by the International Bureau:

This sheet is not part of and does not count as a sheet of the international application.

PCT

FEE CALCULATION SHEET
Annex to the Request

For receiving Office use only

International Application No.

Date stamp of the receiving Office

Applicant's or agent's file reference: H1875 PCT S3

Applicant: GLYCOMED SCIENCES LIMITED

CALCULATION OF PRESCRIBED FEES

1. TRANSMITTAL FEE . . . . . EUR 100.00 [T]

2. SEARCH FEE . . . . . EUR 1,550.00 [S]

International search to be carried out by EPO

(If two or more International Searching Authorities are competent to carry out the international search, indicate the name of the Authority which is chosen to carry out the international search.)

3. INTERNATIONAL FILING FEE

Where items (b) and/or (c) of Box No. IX apply, enter **Sub-total number of sheets**
Where items (b) and (c) of Box No. IX do not apply, enter **Total number of sheets** } 29

[i1] first 30 sheets . . . . . EUR 902.00 [i1]

[i2] _____ (number of sheets in excess of 30) x 10.00 (fee per sheet) = EUR 0.00 [i2]

[i3] additional component (only if sequence listing and/or tables related thereto are filed in computer readable form under Section 801(a)(i), or both in that form and on paper, under Section 801(a)(ii)):

400 x 10.00 (fee per sheet) = EUR [i3]

Add amounts entered at i1, i2 and i3 and enter total at I . . . . EUR 902.00 [I]

(Applicants from certain States are entitled to a reduction of 75% of the international filing fee. Where the applicant is (or all applicants are) so entitled, the total to be entered at I is 25% of the international filing fee.)

4. FEE FOR PRIORITY DOCUMENT *(if applicable)* . . . . . EUR 30.00 [P]

5. TOTAL FEES PAYABLE . . . . . EUR 2,582.00 TOTAL

Add amounts entered at T, S, I and P, and enter total in the TOTAL box

MODE OF PAYMENT

- [X] authorization to charge deposit account (see below)
- [ ] postal money order
- [ ] cash
- [ ] coupons
- [ ] cheque
- [ ] bank draft
- [ ] revenue stamps
- [ ] other *(specify):*

AUTHORIZATION TO CHARGE (OR CREDIT) DEPOSIT ACCOUNT
(This mode of payment may not be available at all receiving Offices)

- [X] Authorization to charge the total fees indicated above.
- [X] *(This check-box may be marked only if the conditions for deposit accounts of the receiving Office so permit)* Authorization to charge any deficiency or credit any overpayment in the total fees indicated above.
- [ ] Authorization to charge the fee for priority document.

Receiving Office: RO/ EPO

Deposit Account No.: 2800.0321

Date: August 8, 2004

Name: Dr. Rudolf Weinberger

Signature:

See Notes to the fee calculation sheet

PCT-Patent Application
based on EP 03 01 5501.4
GlycoMed Sciences Limited
Our Ref.: H1875 PCT S3

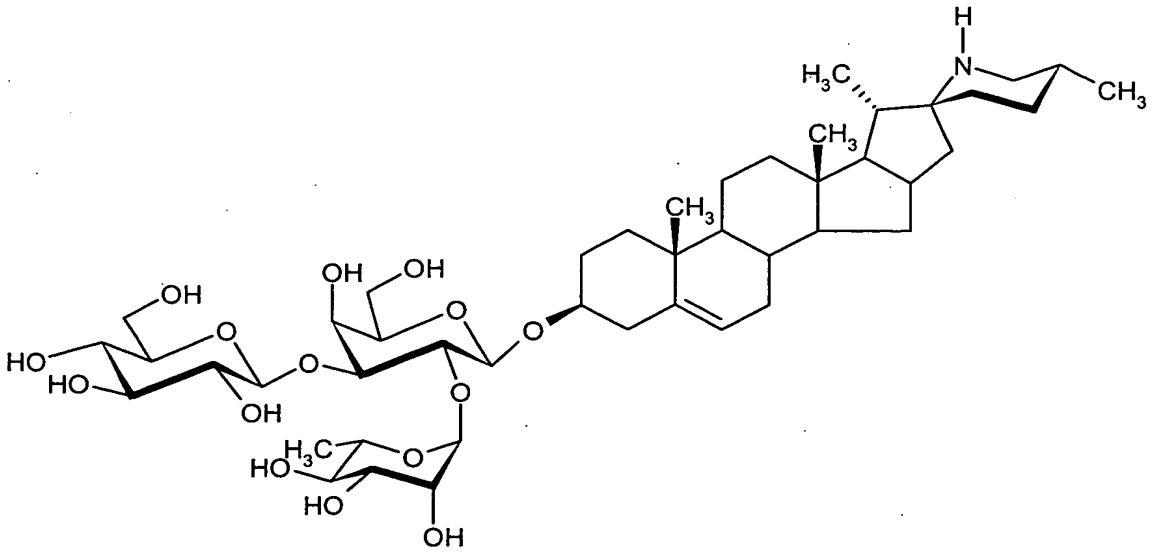
VOSSIUS & PARTNER
PATENTANWÄLTE
SIEBERTSTR. 4
81675 MÜNCHEN

Steroid modified Solatrioses

The present invention relates to the chemical synthesis of alkaloid glycosides, in particular to the synthesis of steroid modified solatrioses. Furthermore, the present invention relates to novel steroid modified solatrioses and intermediate compounds useful for the synthesis thereof.

Solasodine and its glycosides are of considerable interest commercially and clinically. They are widely used as starting products for the synthesis of various steroidal drugs. The aglycon solasodine is a source for synthetic cortisone and progesterone.

It is moreover well established that certain naturally occurring conjugate solasodine glycosides have potent antineoplastic properties. Of particular interest is the triglycoside solasonine (22R, 25R)-spiro-5-en-3β-yl-α-L-rhamno-pyranosyl-(1->2 gal)-O-p-D-glucopyranosyl-(1->3 gal)-β-D-galactopyranose. The structure of this triglycoside is as follows:



Solasonine

The above triglycoside is conventionally obtained by extraction from a plant source. A commercially available extract of *S. sodomaeum*, commonly referred to as BEC

(Drug Future, 1988, vol. 13.8, pages 714-716) is a crude mixture of solamargine, solasonine and their isomeric diglycosides. The extraction process for making BEC involves homogenizing the fruits of *S. sodomaeum* in a large volume of acetic acid, filtering off the liquid through muslin followed by precipitation of the glycosides with ammonia (Drugs of today (1990), Vol. 26 No. 1, p. 55-58, cancer letters (1991), Vol. 59, p. 183-192). The yield of the solasodine glycoside mixture is very low (approx. 1%). Moreover the individual process steps are not defined to GMP in terms of scale up, definition of yield, composition and product quality.

There is a great need for a cost efficient process that provides the antineoplastically active triglycoside solasonine at high yield with little or no impurities.

Contrary to other steroid ring systems, the steroid skeleton of solasodine contains a very labile nitrogen-containing ring. The same holds true for the steroid ring systems of relared alkaloids such as tomatidine, demissidine or solanidine. These aglycons cannot readily be chemically modified while keeping the steroid skeleton intact. In spite of the fact that the aglycon solasodine is readily available, the prior art does not disclose the synthesis of the solasonine using the aglycon material as starting material.

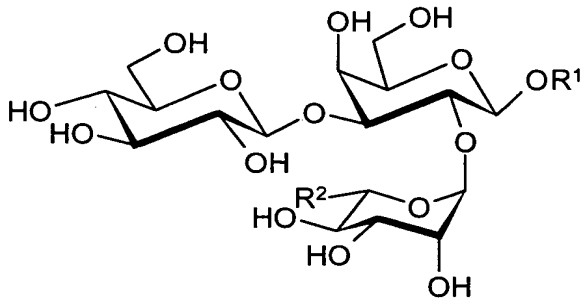
The synthesis of solasonine requires the stereoselective glycosylation of solasodine at the relatively unreactive hydroxyl group.

It has been found that solasodine is not compatible with the conventional steroid glycosylation technique. No glycosylation was observed following the treatment of solasodine with tetrabenzoyl α-D-glucopyranosyl trichloroacetimidate and trimethyl-silyl triflate or boron trifluoride dietherate (unpublished results).

The problem underlying the present invention is to provide a cost effective method for the preparation of solasonine and solasonine analogues in high yields.

Such compounds exhibit cytotoxic activity and may be employed as anticancer agents. Furthermore, such compounds exhibit anti bacterial, anti fungal or anti viral activity.

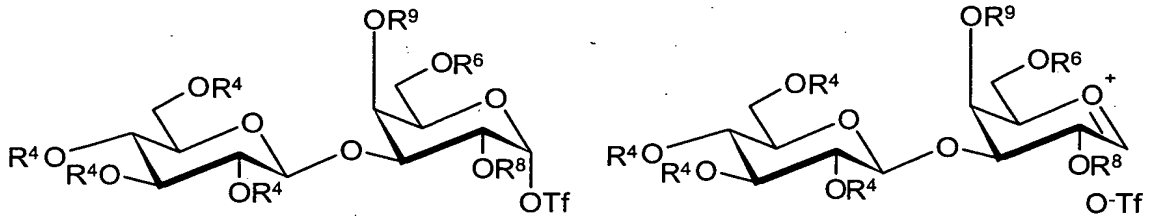
Accordingly, the present invention provides a method for the preparation of a steroid modified solatriose of general formula (I):



Formula (I)

wherein R^1 represents a steroid or a derivative thereof having a hydroxyl group in 3-position and no further unprotected hydroxyl groups; and R^2 represents a straight or branched C_{1-4} alkyl group or a hydroxyl group.

The method of the present invention comprises the step of:
reacting a compound of general formula (XIII):



Formula (XIII)

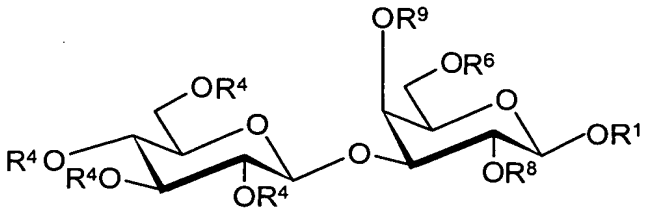
wherein each R^4 independently represents a benzoyl, acetyl or pivolyl protecting group; R^6 represents a pivolyl protecting group; R^8 represents a chloroacetyl protecting group; R^9 represents a benzoyl, acetyl or pivolyl protecting group and Tf represents a triflate leaving group;
with a compound of general formula (XIV):

$$HO–R^1$$

Formula (XIV)

wherein R^1 is as defined above,

to yield a compound of general formula (XV):



Formula (XV)

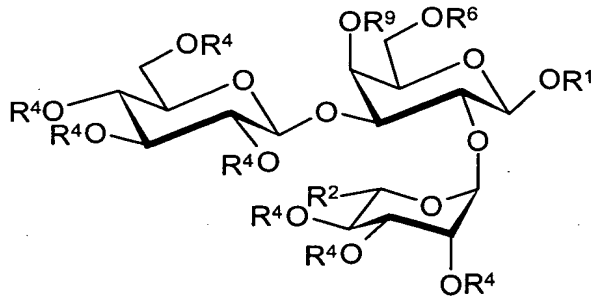
wherein R^1, R^6, R^8 and R^9 are as defined above.

The compound of the above general formula (XV) may be transformed to the desired steroid modified solatriose of general formula (I) by any suitable method known in the art. A particular preferred procedure is described in detail below.

Furthermore, the present application provides steroid modified solatriose compounds of general formula (I) as defined above, wherein R^1 represents a tomatidin-3-yl, demissidin-3-yl, solanidin-3-yl or solasodin-3-yl group.

A further object of the present application is the provision of intermediate compounds useful for the synthesis of the steroid modified solatriose of general formula (I) defined above, namely:

A compound of general formula (XVII):

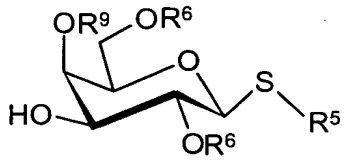


Formula (XVII)

wherein R^1, R^2, R^4, R^6, and R^9 are as defined above.

A compound of general formula (XV) as defined above

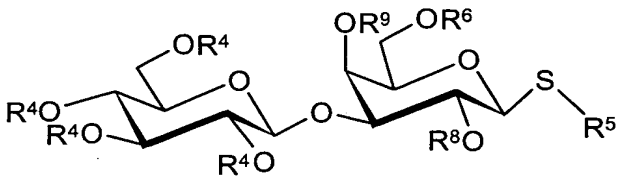
A compound of general formula (X):



Formula (X)

wherein R^6, R^8 and R^9 are as defined above; and R^5 represents a straight or branched C_{1-14} alkyl group or a phenyl group optionally substituted with one or more C_{1-4} alkyl groups, halogen atom such as Cl, F, Br or I, or NO_2 group.

A compound of general formula (XII):



Formula (XII)

wherein R^4, R^5, R^6, R^8 and R^9 are as defined above.

Further embodiments of the present application are described in the dependent claims.

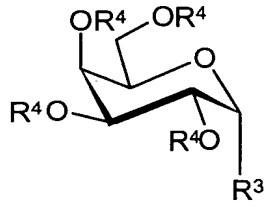
Detailed description of the invention

In the following, the present invention will be explained in more detail with reference to preferred embodiments.

The steroid residue constituting substituent R^1 is a steroid or a derivative thereof having a hydroxyl group in the 3-position for bonding as α-glycosidic hydroxyl group in the compound of general formula (I). The steroid residue bears no further unprotected hydroxyl groups and preferably has no further hydroxyl groups at all, in order not to compromise subsequent reaction steps. In a preferred embodiment of the present invention R^1 is selected from a tomatidin-3-yl, demissidin-3-yl, solanidin-3-yl and solasodin-3-yl group.
All of those steroid groups contain a labile nitrogen-containing ring and, therefore, cannot be chemically modified by means of conventional methods. Moreover, all of the above steroid groups represent substituents for cyctotoxic, anti bacterial, anti fungal or anti viral compounds.

In the above general formula (I) each R^2 independently represents a straight or branched alkyl group having 1 to 4 carbon atoms or a hydroxyl group. In a preferred embodiment, R^2 represents a methyl group.

According to a preferred embodiment of the method of the present invention, galactose is reacted in step (A) to yield a compound of general formula (II):



Formula (II)

wherein R^3 represents a chlorine or bromine atom; and each R^4 independently represents a benzoyl, acetyl or pivolyl protecting group. In a preferred embodiment R^3 represent a bromine atom. In another preferred embodiment R^4 represents an acetyl protecting group.

Step (A) may be carried out using either acetic anhydride, acetyl chloride, benzoyl chloride, benzoic anhydride, or pivolyl chloride in the presence of a base such as, e.g., pyridine, triethylamine, or collidine, to give fully esterified galactose. Esterified-

D-galactopyranose may be treated with hydrogenbromide or hydrogenchloride in glacial acetic acid to yield the above compound of general formula (II).

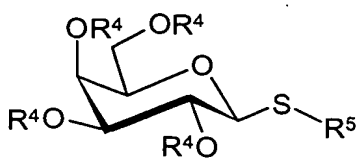
In a particularly preferred embodiment galactose is suspended in organic base such as pyridine and cooled to 0°C, to this solution is added dropwise either acetic anhydride, benzoic anhydride or acid chloride . Upon complete addition the solution is warmed to +25°C (room temperature) and stirred for about 16 hours. The reaction is quenched by addition of alcohol. The solution is diluted with organic solvent such as tert-butylmethyl ether, or dichloromethane, or toluene and washed with cold 1N HCl, water, saturated sodium bicarbonate, water and brine then the product is dried over magnesium sulfate and concentrated under reduced pressure to dryness. The product can be used without further purification or it can be recrystallised.
The fully esterified galactopyranose in dry solvent such as dichloromethane is cooled to O°C under an inert atmosphere. To this solution is added hydrogen bromide in glacial acetic acid, typically 30% HBr content. The solution is allowed to warm to +25°C (room temperature) and stirred for around 16 hours. The solution is diluted with organic solvent such as dichloromethane and then quickly washed with ice cold water, saturated aqueous sodium bicarbonate, and brine. The product is dried over magnesium sulfate filtered and the solvent is removed under reduced pressure. The product is crystallized from petrol (40-60) and diethyl ether.

In step (B), a compound of general formula (II) is reacted with a compound of general formula (III):

$$HS–R^5$$

Formula (III)

wherein R^5 represents a straight or branched C_{1-14} alkyl group or a phenyl group optionally substituted with one or more C_{1-4} alkyl groups; whereby the C1-14 alkyl groups are preferably selected from methyl, ethyl and propyl and the phenyl group is preferably selected form phenyl, p-methylphenyl and p-chlorophenyl; and methyl, ethyl and propyl are particularly preferred;
to yield a compound of general formula (IV):

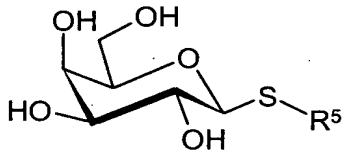


Formula (IV)

wherein R^4 and R^5 are as defined above.

Preferably R^5 is a phenyl group.

Furthermore, in step (C), the compound of general formula (IV) is deprotected to yield a compound of general formula (V):

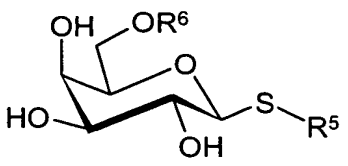


Formula (V)

wherein R^5 is as defined above.

Any suitable deprotection condition conventionally employed in the chemistry of protecting groups may be used. Deprotection is preferably be carried out in an inert organic solvent such as dichloromethane or tetrahydrofuran in the presence of an alkali metal alkoxide having 1 to 4 carbon atoms and a C_{1-4} alcohol, or in the presence of water, an alkali metal hydroxide and a C_{1-4} alcohol. In a particular preferred embodiment deprotection in step (C) is carried out in dry methanol with catalytic amount of sodium methoxide.

Subsequently, the OH group in 6-position is selectively protected in step (D) using a bulky protecting group to yield a compound of general formula (VI)

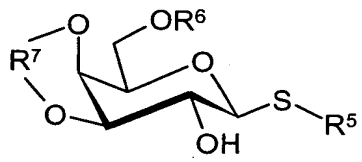


Formula (VI)

wherein R^5 is as defined above; and R^6 is a pivolyl, benzoyl or substituted benzoyl protecting group, whereby the substituents are selected from alkyl groups such as methyl, halogen atoms such as Cl, Br, F,and I and NO_2. Preferably R^6 represents a pivolyl protecting group.

In a preferred embodiment the reaction may be carried out using pivolyl chloride in dry dichloromethane in the presence of pyridine.

In step (E), the OH groups in 3- and 4-position are selectively protected with a ketal or acetal protecting group using standard conditions to yield a compound of general formula (VII):



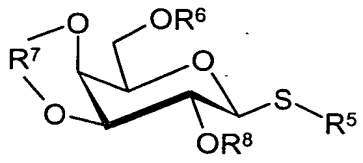
Formula (VII)

wherein R^5 and R^6 are as defined above; and R^7 represents a ketal or acetal type protecting group selected from benzylidene, 4-nitrobenzylidene, 4-methoxybenzylidene or isopropylidene. In a preferred embodiment R^7 represents an isopropylidene protecting group.

The reaction is preferably carried out in a dipolar aprotic solvent such as dimethyl formamide (DMF) or acetone in the presence of acid catalysts such as p-toluene sulfonic acid or camphorsulfonic acid using a 2,2-dialkyloxypropane or an optionally substituted dialkylbenzylidene.

Suitable reaction temperatures range from ambient temperature to elevated temperatures. Preferably the reaction is carried out at a temperature of 25°C.

Moreover, the OH group in 2-position is protected in step (F) by reacting the compound of general formula (VII) with chloroacetyl chloride to yield a compound of general formula (VIII):

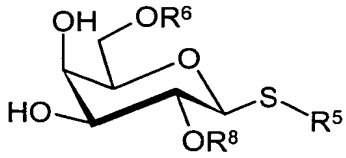


Formula (VIII)

wherein R^5, R^7 and R^8 are as defined above; and R^8 represents a chloroacetyl protecting group.

The reaction may be carried out in a dry solvent such as dichloromethane with a base such as pyridine or triethylamine at a temperature of from 0°C to 25°C.

In step (G) the compound of general formula (VIII) is deprotected to yield a compound of general formula (IX):

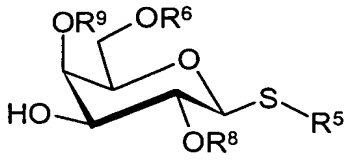


Formula (IX)

wherein R^5, R^6 and R^8 are as defined above.

Deprotection may be carried out under acidic conditions by treating with aqueous acetic acid, aqueous trifluoroacetic acid or mineral or sulfonic acid.

In step (H) the compound of general formula (IX) is reacted with a trialkylorthoacetate, benzoate or pivolate, wherein the alkyl residues have 1 to 4 carbon atoms, to form an 3,4-ortho ester which is subsequently migrated to the axial 4-position under acidic conditions to yield a compound of general formula (X):



Formula (X)

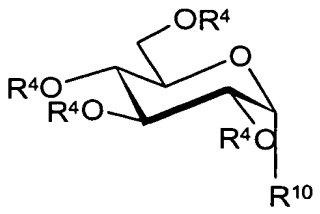
wherein R^5, R^6, R^8 are as defined above and R^9 is an acetyl, benzoyl or pivolyl protecting group. In preferred embodiments R^9 represent an acetate or benzoyl protecting group, which may be introduced by means of trimethyl or triethyl orthoacetate or benzoate, most preferably trimethylorthoacetate.

Step (H) may be conducted in an inert organic solvent such as acetonitrile.

Preferably the reaction is carried out in the presence of a catalyst. Any conventional catalyst used in carbohydrate chemistry may be employed. Particular preferred catalysts include p-toluenesulfonic acid, or camphor sulfonic acid. The most preferred catalyst is p-toluenesulfonic acid.

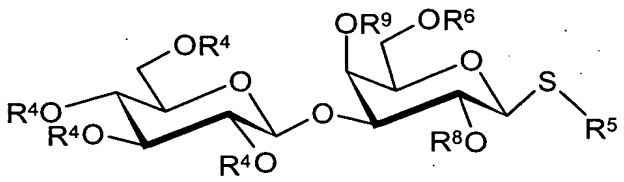
The reaction may preferably be carried out under anhydrous conditions in the presence of a water detracting means such as 4Å mol sieves.

The free OH group in 3-position is reacted in step (I) with a protected halogen glucose derivative of general formula (XI):



Formula (XI)

wherein R^4 is as defined above; and R^{10} represent a halogen atom such as fluorine, chlorine or bromine, to yield a compound of general formula (XII):



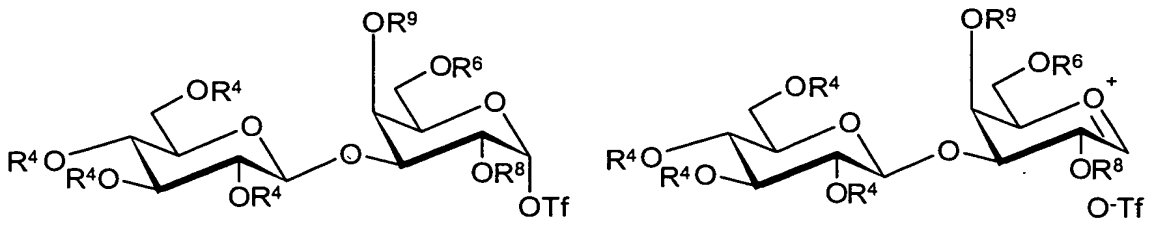
Formula (XII)

wherein R^4, R^5, R^6, R^8 and R^9 are as defined above.

The reaction is preferably carried out in the presence of promoters such as silver triflate, zinc dichloride, borontrifluoride diethyletherate, or N-iodosuccinamide/triflic acid.

In a preferred embodiment a dry solvent such as dichloromethane is employed. The reaction temperature is preferably at a range of from –20°C to 25°C.

Activating compound (XII) may be achived in step (J) through the oxidiation of the thio ether to the sulfoxide and the formation of the anomer trifate of general formula (XIII) below, which may exist as either the alpha triflate or the alpha ion pair:



Formula (XIII)

wherein R^4, R^5, R^6, R^8 and R^9 are as defined above.

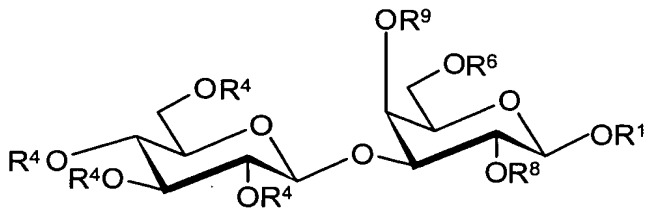
The reaction is preferably carried out by oxidizing the thio ether group to a sulfoxide using hydrogen peroxide, and subsequently treating the resulting intermediate with triflic anhydride. Furthermore, in a particular preferred embodiment, a sterically hindered non-nucleophilic base such as 2,6-lutidine, 2,4,6-collidine or 2,6-di-tertbutyl-4-methyl-pyridine is present. The most preferred sterically hindered base is 2,6-di-tertbutyl-4-methly-pyridine.

In step (K), coupling of the compound of general formula (XIII) with the compound of general formula (XIV)

$HO\text{-}R^1$

Formula (XIV)

wherein R^1 is as defined above; may be performed in the presence of sterically hindered non-nucleophilic base such as 2,6-lutidine, 2,4,6-collidine or 2,6-di-tertbutyl-4-methyl pyridine, preferably 2,6-di-tertbutyl-4-methyl-pyridine, to yield a compound of general formula (XV):



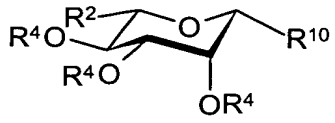
Formula (XV)

wherein R^1, R^6, R^8 and R^9 are as defined above.

The reaction may preferably be carried out under anhydrous conditions in the presence of a water detracting means such as 4Å mol sieves.

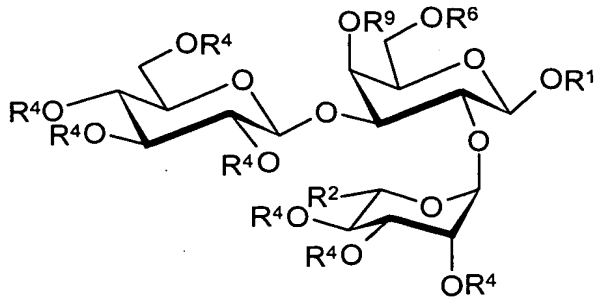
In a preferred embodiment the reaction is carried out at low temperature such as 0°C or lower, more preferably –10°C or lower. The most preferred reaction temperature is –20°C.

In step (L), the OH group in 2-position substituted with R^8 is selectively deprotected using thio urea in the presence of a sterically hindered base such as 2,6-lutidine, 2,4,6-collidine or 2,6-di-tertbutyl-4-methyl pyridine, preferably 2,6-lutidine, in a dry alcohol such as methanol, ethanol or isopropanol, preferably ethanol, and subsequently reacted with a protected halogen rhanmose derivative of general formula (XVI):



Formula (XVI)

wherein R^2 and R^4 are as defined above; and R^{11} represents a halogen atom such as bromine, chlorine or fluorine, preferably bromine, to yield a compound of general formula (XVII):



Formula (XVII)

wherein R^1, R^2, R^4, R^6, and R^9 are as defined above.

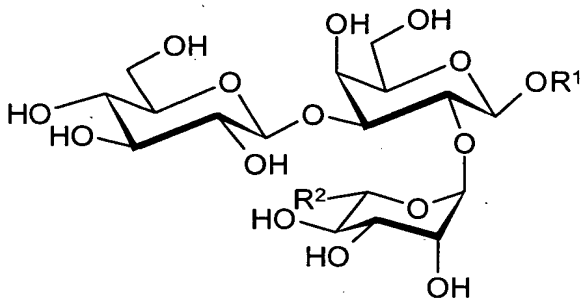
The deprotection in step (M) may be performed under substantially the same conditions as described above for step (C) to yield the compound of general formula (I). In a preferred embodiment, deesterification may by accomplished using sodium methoxide in a methanol/dichloromethane mixture.

Abstract

The invention pertains to steroid modified solatrioses and the synthesis thereof as well as to intermediate compounds useful for the synthesis of the steroid modified solatrioses.

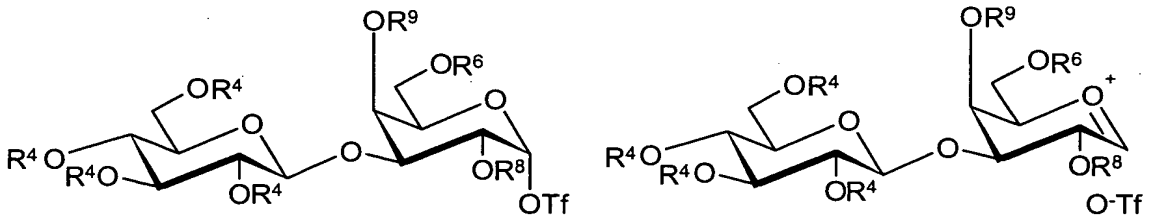
Claims

1. A method for the preparation of a steroid modified solatriose of general formula (I):



Formula (I)

wherein R^1 represents a steroid or a derivative thereof having a hydroxyl group in 3-position and no further unprotected hydroxyl groups; and R^2 represents a straight or branched C_{1-4} alkyl group or a hydroxyl group,
which method comprises the step of:
reacting a compound of general formula (XIII):



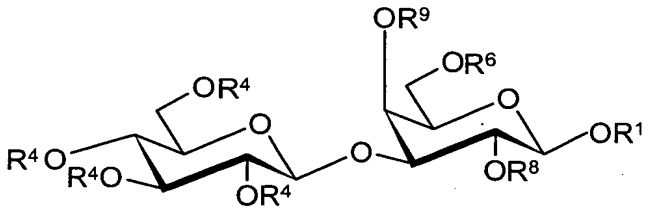
Formula (XIII)

wherein each R^4 independently represents a benzoyl, acetyl or pivolyl protecting group; R^6 represents a pivolyl protecting group; R^8 represents a chloroacetyl protecting group; R^9 represents a benzoyl, acetyl or pivolyl protecting group; and Tf represents a triflate leaving group;
with a compound of general formula (XIV):

$$HO–R^1$$

Formula (XIV)

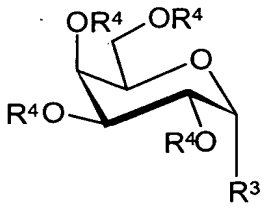
wherein R^1 is as defined above
to yield a compound of general formula (XV):



Formula (XV)

wherein R^1, R^6, R^8 and R^9 are as defined above.

2. The method according to claim 2, further comprising the step of:
reacting galactose to yield a galactose fully protected with ester type protecting groups, and subsequently treating with hydrogen bromide or hydrogen chloride to yield a compound of general formula (II):



Formula (II)

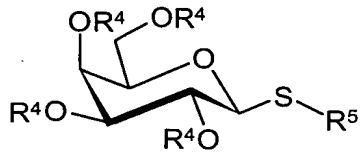
wherein R^3 represents a chlorine or bromine atom; and R^4 is as defined in claim 1.

3. The method according to claims 1 or 2, further comprising the step:
reacting a compound of general formula (II) as defined in claim 2, with a compound of general formula (III):

$$HS–R^5$$

Formula (III)

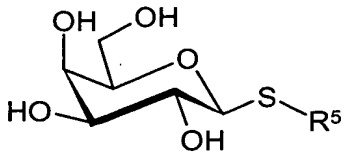
wherein R^5 represents a straight or branched C_{1-14} alkyl group or a phenyl group optionally substituted with one or more C_{1-4} alkyl groups whereby the C1-14 alkyl groups are preferably selected from methyl, ethyl and propyl and the phenyl group is preferably selected form phenyl, p-methylphenyl and p-chlorophenyl; and methyl, ethyl and propyl are particularly preferred;
to yield a compound of general formula (IV):



Formula (IV)

wherein R^4 is as defined in claim 1, and R^5 is as defined above.

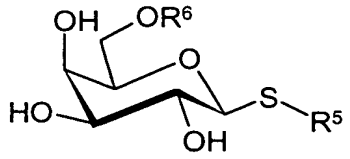
4. The method according to any of claims 1 to 3, further comprising the step of:
deprotecting a compound of general formula (IV) as defined in claim 3 to yield a compound of general formula (V):



Formula (V)

wherein R^5 is as defined in claim 3.

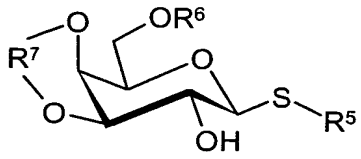
5. The method according to any of claims 1 to 4, further comprising the step of: selectively protecting the OH group in the 6-position of a compound of formula (V) as defined in claim 4 with pivolyl chloride using standard conditions to yield a compound of general formula (VI):



Formula (VI)

wherein R^5 in claim 3; and R^6 is a pivolyl, benzoyl or substituted benzoyl protecting group, whereby the substituents are selected from alkyl groups such as methyl, halogen atoms such as Cl, Br, F, and I and NO_2 .

6. The method according to any of claims 1 to 5, further comprising the step of: selectively protecting the OH groups in 3- and 4-position with a ketal or acetal protecting type protecting group using standard conditions, to yield a compound of general formula (VII):

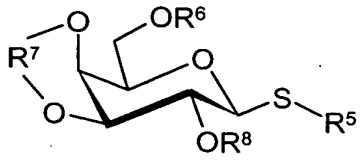


Formula (VII)

wherein R^5 and R^6 are as defined in claims 3 and 5, respectively; and R^7 represents a ketal or acetal type protecting group selected from the group consisting of benzylidene, 4-nitrobenzylidene, 4-methoxybenzylidene and isopropylidene.

7. The method according to any of claims 1 to 6, further comprising the step of:

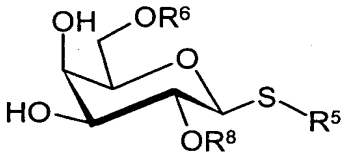
protecting the OH group in 2-position of the compound of general formula (VII) as defined in claim 6 with chloroacetyl chloride using standard conditions, to yield a compound of general formula (VIII):



Formula (VIII)

wherein R^5, R^6 and R^7 are as defined in claims 3, 5 and 6, respectively; and R^8 represents a chloroacetyl protecting group.

8. The method according to any of claims 1 to 7, further comprising the step of: selectively deprotecting the OH group in 3- and 4-position of the compound of general formula (VIII) as defined in claim 7 using standard conditions, to yield a compound of general formula (IX):

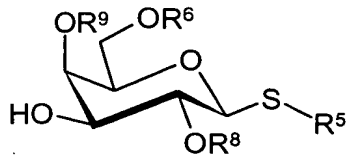


Formula (IX)

wherein R^5, R^6, and R^8 are as defined in claims 3, 5 and 7, respectively.

9. The method according to any of claims 1 to 8, further comprising the step of: reacting the compound of general formula (IX) with a trialkylorthoacetate, benzoate or pivolate to form an 3,4-orthor ester which is subsequently

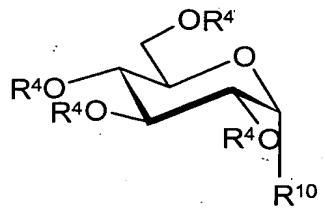
migrated to the axial 4-position under acidic conditions to yield a compound of general formula (X):



Formula (X)

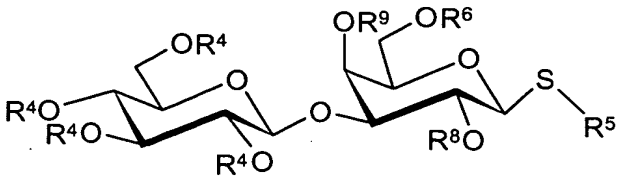
wherein R^5, R^6, R^8 and R^9 are as defined in claims 3, 5, 7 and 1 respectively.

10. The method according to any of claims 1 to 9, further comprising the step of: reacting the OH group in 3-position of the compound of general formula (X) as defined in claim 9 with a protected halogen glucose derivative of general formula (XI):



Formula (XI)

wherein R^4 is as defined in claim 1; and R^{10} represent a halogen atom, a trichloroacetimidiate group, or a thioalkyl group having 1 to 14 carbon atoms, to yield a compound of general formula (XII):

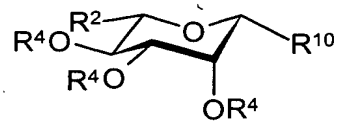


Formula (XII)

wherein R^4, R^5, R^6, R^8 and R^9 are as defined in claims 1, 3, 5, 7 and 9, respectively.

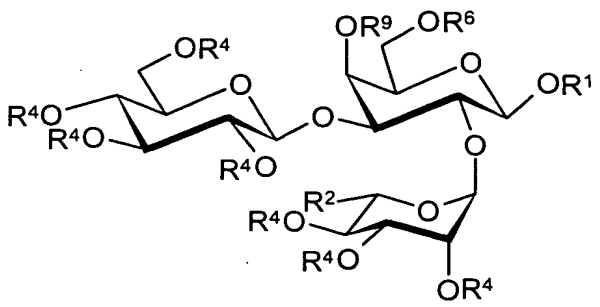
11. The method according to any of claims 1 to 10, further comprising the step of: activating the compound of general formula (XII) as defined in claim 10 by oxidizing the thio ether group to a sulfoxide using hydrogen peroxide, and subsequently treating the resulting intermediate with triflic anhydride, to yield a compound of general formula (XIII) as defined in claim 1.

12. The method according to any of claims 1 to 13, further comprising the step of: selectively deprotecting the OH group in the 2-position of the compound fo general formula (XV) as defined in claim 1 using thio urea in the presence of a sterically hindered non-nucleophilic base, and subsequently reacting the resulting intermediate with a protected halogen rhanmose derivative of general formula (XVI):



Formula (XVI)

wherein R^2, R^4 and R^{10} are as defined in claims 1 and 10, respectively; to yield a compound of general formula (XVII):



Formula (XVII)

wherein R^1, R^2, R^4, R^6, and R^9 are as defined in claims 1, 5 and 9, respectively.

13. The method according to any of claims 1 to 12, further comprising the step of: deprotecting the compound of general formula (XVII) as defined in claim 12, to yield the compound of general formula (I) as defined in claim 1.

14. The method according to any of the preceding claims, wherein R^1 represents a tomatidin-3-yl, demissidin-3-yl, solanidin-3-yl and solasodin-3-yl group.

15. The method according to claims any of the preceding claims, wherein R^2 represents a methyl group.

16. The method according to any of the preceding claims, wherein R^3 in the compound of general formula (II) represents a bromine atom.

17. The method according to any of the preceding claims, wherein R^4 in the compound of general formula (II) represents an acetyl protecting group.

18. The method according to any of the preceding claims 1, wherein R^5 in the compound of general formula (III) represents a phenyl group.

19. The method according to any of the preceding claims, wherein R^7 in the compound of general formula (VII) represents a isopropylidene protecting group.

20. The method according to any of the preceding claims, wherein R^4 in the compounds of general formula (XI) and/or compound of general formula (XVI) represents a benzoyl protecting group.

21. The method according to any of the preceding claims, wherein reacting a compound of general formula (XIII) with a compound of general formula (XIV) is carried out in the presence of sterically hindered non-nucleophilic base.

22. The method according to claim 21, wherein the sterically hindered non-nucleophilic base is selected from 2,6-lutidine, 2,4,6-collidine or 2,6-di-tertbutyl-4-methyl pyridine.

23. A steroid modified solatriose of general formula (I) as defined in claims 1 or 15, wherein R^1 represents a tomatidin-3-yl or demissidin-3-yl group.

24. A compound of general formula (XVII) as defined in claims 12 or 15.

25. A compound of general formula (XV) as defined in claims 1 and 15.

26. A compound of general formula (X) as defined in claim 9.

27. A compound of general formula (XII) as defined in claim 10.